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IN ETHANOLIC BUFFERS

by

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ABSTRACT

The contribution of scattering to the circular dichroism (CD) of DNA films with twisted structures, DNA-polylysine complexes, and condensed DNA aggregates in ethanolic buffers of defined salt concentrations has been studied by the use of novel measuring techniques. These techniques include Fluoroscat cuvettes, fluorescence detected circular dichroism (FDCD) methods, back scattering capturing devices and beam mounted goniometer detectors. The result of the experimental measurement is that DNA films can be made which have very large ellipticities or CD at sharp specific wavelengths. The sign of these ellipticities is related to the handedness of the twists, with a right handed twist producing large positive rotations, and a left handed one producing negative rotations. The film shows nodal angles at which the interaction with light is minimal. The scattering patterns of both films, DNA-polylysine particles, and DNA-EtOH condensates show that the main interaction is light scattering produced by a resonance phenomenon similar to that produced in cholesteric liquid crystals and twisted nematic liquid crystals. From this result we propose that the so-called psi type CD spectrum is a manifestation of a side by side
packing of DNA molecules with a long range twisting order whose helical parameters match the helical parameter of circularly polarized light at specific resonance or critical wavelengths. Application of the Bragg law for cholesteric liquid crystals gives the periodicity of the long range ordered structures.
INTRODUCTION

As can be seen in the first paper in this series (Reich et al., 1979) the perturbations due to scattering of the CD signals on a variety of particles can be divided into two general types: a) that signal which can be corrected by recapturing the light deviated away from the detection system and b) that CD signal which is not corrected by increasing the efficiency of light collection and which may even be enhanced by increasing the envelope of light capture and/or the efficiency of measurement of the CD signal about the scattering particle.

The first type of perturbation is the best understood (Holzwarth et al. (1974), Gordon (1972), and Gordon and Holzwarth (1971). Reasonable approximations to certain types of optically active particles by the application of Mie scattering theory of a sphere have had a measure of success in explaining the absorbance spectrum and some of the so-called Duysens flattening manifested by large membranes. (Schneider and Harmatz, 1976). However, it was found in the case of bacteriophage T2 that the Mie theory was not successful in predicting the CD of the condensed nucleic acids inside the head of the virus (Holzwarth et al., 1974). In fact, the CD scattering components of the intact virus as measured by Dorman and Maestre (1973) show properties very similar to those measured in the optical activity dispersion curve of cholesteric liquid crystals (Holzwarth et al., 1974).

The optical properties of liquid crystals with a well-defined chiral structure (i.e. cholesteric liquid crystals and twisted nematic liquid crystals) are indeed remarkable in their nature. They have been extensively studied (see de Gennes, 1974 and Chandrasekhar, 1977 for
excellent treatises on the analysis of the optical properties of liquid crystals) and since these optical properties are produced by a phenomenon of macroscopic dimensions they can be easily analyzed by the use of macroscopic dielectric tensors in combination with Jones matrix calculus and Stokes vectors (see Shurcliff "Polarized Light" and Chandrasekhar 1977). We shall mention some of the most pertinent properties as follows:

1. Cholesteric liquid crystals have bands at critical wavelength at which strong interactions occur with impinging light of the correct polarization. That is, a cholesteric liquid crystal of right handed helical structure will interact strongly with a right circularly polarized wave impinging upon it if the periodicity of the wave matches that given by a Bragg reflection law, to wit Eq. 1. \( n\lambda = 2L \cos r' \) (de Gennes, Chapter 6, 1974) where \( n \) = order, \( \lambda \) = wavelength, \( L \) = repeat period, \( r' \) = internal angle of refraction.

2. If at that wavelength there exist chromophores absorbing light, the phenomenon of anomalous transmission occurs (Nitayananda, 1973 and Luresh, 1976) in which an apparent excess transmission or loss of absorbers is measured. This is analogous to the Borrmann effect (1941) for x-ray diffraction when a crystal is set for Bragg reflection.

3. The polarization of the reflected waves is the same as that of the impinging wave, which is the opposite case to that of ordinary reflection in which there is a phase shift of 180°. Thus, for a cholesteric array at the critical or resonance wavelength, the scattered light has the same sense of polarization as the incoming
wave. However, it reflects the helical sense of the scattering structure and it is related to the pitch by Eq. no. 1. In this property it also mimics the scattering behavior of an array of helical wire antennas for circularly polarized radio waves (Krauss' Antennas, 1950). These properties were used by Freeman and Tinoco (1957) to model the interaction of light with optically active chromophores by the use of arrays of wire coils and a radar transmitter with detectors in the 1.4 cm wavelength range.

4. The most remarkable property of all is the strength of the interaction. The rotational strengths measured for cholesteric liquid crystals or twisted nematics are $10^4$ times (i.e. four orders of magnitude) larger than that of the optically active components. This is because of the very strong resonance coupling with the light beam at the critical wavelength. Thus the large magnitude of the CD signals provide a strong clue to the explanation for the so-called psi type (ψ) spectra measured in DNA aggregates or particles (Jordan et al., 1972).

ψ(±) type CD spectra have been reported with very large specific circular dichroism in films of DNA by Tunis-Schneider and Maestre (1970) and by Brunner and Maestre (1974). Magnitudes for $\varepsilon_\lambda - \varepsilon_\tau = -300$ for films of poly [d(A-T)·d(A-T)] and values as large as -70 for films of calf thymus DNA have been measured by these workers. Large values have also been reported for polylysine-DNA aggregates ($\Delta \varepsilon = -28$) and polyuridine-polylysine complexes or aggregates, ($\Delta \varepsilon = +120$) by Carroll (1972). The paper preceding this one showed very large values for DNA-particles produced by specific concentrations of salt-EtOH buffers.
What is most remarkable about these nucleic acid complexes, films, and aggregates is that the large values are not corrected by large angle integrating sphere methods and in the case of the DNA-EtOH particles the magnitudes are substantially increased. The scattering components of the CD of the particles that are correctable by FDCD or fluorescent methods also show remarkable directional properties which are not typical of the ordinary scattering behavior of non-optically active particles and of some optically active particles. For DNA-EtOH-salt condensates or particles the main CD (correctable) scattering components are at right angles to the incoming light beam and also in the back direction with very little measured in the forward scattering cone.

All the above-mentioned experimental characteristics lead us to believe that what we are measuring are manifestations of optically active behavior of long range order of the type seen in cholesteric or twisted nematic liquid crystals or alternatively that of helical antennae arrays.

Thus we first attempted to develop cholesteric or twisted nematic films of DNA and then proceeded to measure the reflection or resonance phenomena or scattering patterns by novel experimental techniques. In the next section we will describe in detail how we measured every component, and its direction, of DNA films of defined helical superstructure, DNA-polylysine complexes of varying (DNA/polylysine) ratios and of ethanol condensed DNA, or particles, of extremely large optical rotations or dichroism.

We shall show by the use of these techniques and materials that
psi type spectra, both positive and negative, are a manifestation of superorganization of the DNA in these particles, films, and aggregates. Moreover, these CD signals occur at specific wavelengths reflecting the repeat periodicity of the helical superstructures and that such periodicities are related to the maximum and minimum CD signals by some type of reflection Bragg scattering law.
TECHNIQUES AND METHODS

We shall describe in some detail the variety of techniques used in measuring the optical interaction of films, DNA-polylysine particles and ethanolic particles. Some of these techniques have been reported previously, having been used in the measurement of scattering optically active particles (Dorman and Maestre, 1973, Dorman et al., 1973). It is important to understand how and precisely what each of these techniques is measuring and how the signal from the CD machine must be further processed to obtain the true intensity and polarization values for the light after interaction with the scattering object.

1. Circular Dichroism as measured by the Grosjean and Legrand (1960) technique. This technique uses the modulation of linear polarized light by a quarter wave plate to which is applied an oscillating variable voltage (Pockels' cell or electrooptic modulator) thereby rotating its optical axes by 90°, back and forth, producing a positive or negative 90° phase shift on the x, y components of the incoming light beam. The result is that circularly polarized light is produced, first with one sense of polarization, followed by the opposite sense (i.e., left circularly polarized light followed by right circularly polarized). There are several ways of producing this effect including mechanical rocking of the λ/4 plate or the use of piezoelectric modulators (Morevue modulator). The circularly polarized light of intensity I₀ interacts with the material under investigation and after the interaction a quantity I is measured. The usual circular dichrograph, following the design of Grosjean and Legrand (1960), obtains by electronic means a ratio of intensities
If the material in question obeys a Beer-Lambert absorption law for each polarization we have for

\[ I_\lambda = I_{0\lambda} e^{-c\varepsilon_\lambda d}, \quad I_r = I_{0r} e^{-c\varepsilon_r d} \]

Eq. 3

and signal \( = k \cdot \tanh (\varepsilon_\lambda - \varepsilon_r) \)

\[ \text{Signal} = k \cdot (\varepsilon_\lambda - \varepsilon_r) \] for ellipticities of the order of 5° or less.

\[ = k \cdot \Delta \varepsilon \]  

Eq. 4

\( \varepsilon_r \) = extinction coefficient for right circularly polarized light

\( \varepsilon_\lambda \) = extinction coefficient for left circularly polarized light

The above equation implies pure absorption phenomena. No scattering (anisotropic or otherwise), fluorescence or phosphorescence is assumed, and the measuring instrument is perfect (i.e., no stray light, linear birefringence in optics or detectors or linear dichroism or circular dichroism in the optics or detectors is assumed).

For the case in which a particle large enough to produce long range effects among the interacting chromophores, for a given range of wavelength, the above equations must be modified to include these perturbations as follows:

\[ \text{Signal} = k \cdot \frac{(I_\lambda + S_\lambda) - (I_r + S_r)}{(I_\lambda + S_\lambda) + (I_r + S_r)} \]

Eq. 5

\( k \) = instrument constant and \( S_\lambda \) = scattered left circularly polarized
light $S_r = \text{scattered right, etc.}$

The terms $S_L, S_R$ can be defined formally to include: 
a) scattered light deviated isotropically from the main direction of the beam (ordinary scattering, $S_L = S_R$, $S_r > 0$), 
b) scattered light deviated anisotropically (i.e., differential circular dichroism scattering). Linear dichoric scattering can also occur and can be of importance in film work later. ($S_L \neq S_R$, $S = \text{function of orientation angles with respect to light beam}$.)

3. Stray light contributions from cuvette window reflections, wall reflections, dust in solutions and in cuvette windows and reflections from the optics.

4. The signal can also include non Beer-Lambert contributions from such particles whose effective extinction is almost total, in which case the effect is manifested as a Duysens flattening distortion for the circular dichroism signal. This has been seen in membranes (Schneider, 1973) whole red blood cells (Gitter-Amir et al., 1976) and in crystals of protein (in this last case reversibly) (Chiu and Maestre, in preparation).

5. Most importantly, the signal can include enhancement scattering due to a manifestation of anomalous transmission. $S_L \neq S_R$ and $S_L$ or $S_R$ having a positive value so as to make it appear that less absorption occurs for a given set of chromophores (i.e., anisotropic hypochromicity) for a particular polarization and region of the spectrum. (See Chandrasekhar, Liquid Crystals 1977).

The above equations describe the case in which the detection system is centered about the incoming light beam and also measures the
incoming light plus all scattering components that lie within its acceptance measuring cone.

If the technique requires measurements outside the main beam (i.e., CD as a function of scattering angle by a beam mounted goniometer detector system) the terms I disappear and we have now

$$\text{Signal} = k \cdot \Delta \theta \cdot \frac{S_L(\theta) - S_R(\theta)}{S_L(\theta) + S_R(\theta) + \text{stray light components}}$$

Eq. 6

$\Delta \theta =$ angle of acceptance of detection system.

Note: at a given angle $\theta$ the total scattering $S_L(\theta) + S_R(\theta)$ can be very small so that the stray light can make quite a large contribution. Conversely, if both stray light and total scattering are small at a given angle $\theta$ we may have a very large apparent CD at that angle. Thus the interpretation of these types of measurements should be done with caution and should be checked by differing techniques as we did in our measurements.

6. For the measurement of the CD of films and the scattering CD components the equations now become functions of the angles of incidence $i$ (tilt angle), the angle of internal refraction $r$ and the orientation angle about the axis of the incoming light beam as shown in Fig. 1a. It has been shown by Tunis-Schneider and Maestre (1970) that a film with linear orientation will introduce an apparent circular dichroism component to the intrinsic CD as given by

$$CD_{\text{apparent}} = CD_{\text{intrinsic}} - 0.298 p \cos 2\phi$$

Eq. 7

where $p =$ linear dichroism and $\phi =$ angle of rotation about the optical axis.
Therefore, it is important when measuring the CD of films that have been manipulated in their manufacture to check for possible linear dichroism components by rotating the film assembly about the optical axis.
NOMENCLATURE AND DESCRIPTION OF TECHNIQUES

1. Small angle of acceptance circular dichroism ("normal CD"). Acceptance angles range between 3° to 5°. This is the standard CD machine configuration.

2. Large angle acceptance CD. This is usually measured with a large 2" diameter end window PM tube (Dumont no. 2706 or Hamamatsu R375) especially selected for low birefringence artifacts. The angle of acceptance is increased by bringing the tube close to the cuvette (see Dorman et al. (1973) for a complete discussion of this technique).

3. Fluorescence cuvette integrating methods. As described by Dorman et al (1973), these are two cuvettes, one inside the other. The outer one is filled with a fluorescent solution whose main absorbance band covers the region of the spectrum of interest, in this case sodium salicylate in absolute ethanol. The mechanism of correction utilizes the fact that all light that is not absorbed in the inner chamber eventually hits a fluorescent molecule in the outer chamber. What the photomultiplier sees is the fluorescence emission due to excitation by this light. The fluorescence cuvette covers most of the scattering envelope except for a region towards the back where the light beam impinges upon the first window of the inner cuvette containing the material of interest.

4. Fluorescence Detected Circular Dichroism (FDCD) (See Fig. 1b). As shown in the preceding article, this technique, properly used, corrects for all the differential scattering including the back scattering cone. It is probably the most efficient means of recovering information scattered in all directions. An interesting modification of this method is the one described in Fig. 1c where a DNA film is sandwiched
between two fluorescence cuvettes, a back cuvette covering the back scattering cone and a front cuvette measuring the front scattering cone. This technique allows the individual and separate measurement of these two regions of the scattering envelope. This technique was used to measure the back scattering lobes of the DNA films, the DNA-EtOH condensates, and the DNA-polylysine aggregates.

5. A similar technique is shown in Fig. 1d where a front-surface mirror recovers a large part of the back scattered light and reflects it into the detector.

6. The beam goniometer mounted detector (Fig. 1a) provides information on the CD scattering as a function of scattering angle. The scattering acceptance angle is defined by the width of the slits in front of the photomultiplier tube.

7. Vertical assembly and tilt angle device. The manufacture of films that are extremely wet implies that the film has to lie parallel to the horizontal until it is reasonably rigid. To measure the CD of these wet films and the tilting angle studies we used the instrument developed for CD film studies by Maestre (1970). This device allows the rotation of the film assembly about the light beam (rotation of \( \phi \), Fig. 1a) in order to study linear dichroism artifacts and the detection of "null" scattering angles \( \theta \) by tilting.
PREPARATION OF THE FILMS

This is an art form and practice makes perfect. For example, calf thymus DNA is placed in a totally dehydrated lyophilized form on top of the bottom fused silica window. Distilled water or buffer is added drop by drop until all the DNA is submerged under a solution droplet. After the DNA fibers have swollen and gone into solution to form a very viscous liquid they are covered very gently by the top quartz plate and then pressed down with the proper twist (counterclockwise or clockwise). This twist is small - only about 45° to 90° rotation initially. Then the film is placed in the vertical assembly and its CD is measured. Usually the rotations are so large that the maxima of the CD peaks cannot be measured in the Cary 60 CD machine (2° maximum scale). The film is then squeezed some more to reduce the thickness enough to bring the measurement on scale. Keep trying! After measuring, the twist is then reversed until an opposite CD is obtained (usually about 90° to 180° twist) and so forth. The films usually have an optical density at 260 nm of between 0.3 to 0.7. Usually about four to five reversals can be done before the films become so disoriented that one observes the ordinary CD of DNA (conservative CD spectra). One must guard against introducing linear orientation components and this is checked by rotating the whole film assembly by 90°.
PREPARATION OF THE T7 PHAGE DNA-POLYLYSINE SCATTERING COMPLEXES
\(\psi(-)\) TYPE PARTICLES

_0.85M and 1M salt._ A solution of T7 phage DNA was obtained by phenol extraction from intact T7 bacteriophage and then dialyzed into 1.5M NaCl, 0.01 sodium cacodylate, pH 7.0. Poly-L-lysine hydrobromide (VIIB) was purchased from Sigma Biochemicals. This was reported to have a molecular weight of 32,000 and a DP of 150 by the supplier. A sample of the polylysine was submitted to the microanalytical laboratory of the chemistry department, University of California, Berkeley, for an elemental analysis in order that accurate solution concentrations of the polynucleotide could be obtained. Solutions were made up in 1.5M NaCl, 0.01 M sodium cacodylate, pH 7.0 and added to the above DNA solution so that a final 1:1 ratio of nucleotide to lysine residue was obtained. In 1.5M salt, polylysine and DNA do not form particles while at lower salt concentrations, in which the two species do condense, a 1:1 ratio of lysine to DNA base is observed (Shapiro et al., 1969). The DNA, polylysine mixture was then dialyzed into 1M NaCl, 0.01 sodium cacodylate, pH 7.0, Shapiro et al. (1969) reported that at 0.85M to 1.0M NaCl, the salt concentration was at an optimum for the formation of reversible particles having \(\psi(-)\) DNA character.

_0.15M salt solutions._ Two solutions of T7 DNA: polylysine in 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0 were prepared using differing methods. Solution No. 1 was prepared by dialyzing the DNA, polylysine mixture into 0.85M NaCl and then into 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0. Solution No. 2 was prepared by dialyzing the DNA, polylysine mixture into 1.0M, 0.7M, 0.4M and finally 0.15M NaCl, 0.01M sodium cacodylate, pH 7.0. Both types of particles showed the CD characteristics of \(\psi(-)\) type particles.
E. coli DNA-polylysine complexes. E. coli DNA was obtained from Worthington Biochemicals. A solution was made up in 1.5M NaCl, 0.01M sodium cacodylate, pH 7.0 and run through a 23 gauge needle 11 times in order to reduce the size of the DNA molecules. The polylysine was then added so that a final 1:1 ratio of lysine to nucleotide was obtained. \( \psi(-) \) DNA particles were produced by dialysing the polylysine, DNA mixture vs. 1.0M NaCl and 0.80M NaCl, 0.01M sodium cacodylate, pH 7.0. In this case the salt concentrations were the same as those of Shapiro et al. (1963).

E. coli DNA ethanolic condensates. E. coli DNA solution was placed in 80% ethanol-buffered solutions as reported previously (Reich et al.). This particular preparation was highly concentrated, however, with an optical density value of 10 at 260 nm, prior to the addition of the ethanol. With this method most of the DNA is precipitated. The precipitated material is concentrated in a high speed centrifuge after evaporating the ethanol off under vacuum. The DNA is then redissolved in buffer and the addition of ethanol to 80% w/w is repeated. In this manner DNA-ethanolic \( \psi(+) \) particles with very large positive ellipticities are obtained. (Fig. 14).

Computations of scattering Components

Several modifications have to be introduced in the FDCD equations to allow computation of the back scattering or resonance reflection intensities for each polarization. The appendix shows the equations for the following cases:
i) Film with no scattering components gives with both cuvettes filled in Fig. 1(c)

\[ \theta_F = (14.32)(2.303) \frac{A}{(10^A + 1)} \]

\[ \Delta A = \frac{\theta_F(10^A + 1)}{32.98} \]

ii) Film scattering all in the back direction, both cuvettes filled in Fig. 1(c)

\[ \Delta A = \frac{\theta_F(10^A + 1)}{32.98} \]

iii) Film with scattering all in the back direction with the back cuvette full of fluorescer. Fig. 1(c)

\[ \theta_F = -14.32 \frac{\Delta A_s}{A_D} (10^{-AF} - 10^{-A}) \]

\[ \Delta A_s = \frac{-\theta_F A_D}{14.32(10^{-AF} - 10^{-A})} \]

where

\[ \Delta A_s = \text{Differential back scattering component or resonance reflection (see Appendix)} \]

\[ A_D = \text{Absorption of film} \]

\[ A_F = \text{Absorption of fluorescer in back cuvette} \]

\[ A = \text{Total absorption and scattering extinction of complete system} \]

These are the equations used in computing the components obtained by the method described in Fig. 1(c).
RESULTS

Film experiments. Figures 2 to 6 show the results of a variety of techniques employed in measuring the interaction with circularly polarized light. Fig. 2 displays the circular dichroism as a function of twist. The magnitudes at the maximum are $\Delta \varepsilon$ (right handed twist) = $+120$ at 293 nm and $+100$ at 233 nm. When the film is twisted clockwise (i.e., a left handed twist) we observe $\Delta \varepsilon$ (left handed twist) = $-210$ at 280 nm and $\Delta \varepsilon = -169$ at 222 nm. These are enormous values for the CD of DNA. DNA in solution has values on the order of $\Delta \varepsilon = +2.2$ at 275 nm and $\Delta \varepsilon = -3.3$ at 245 nm. The CD is shown to be reversed once more by again twisting counterclockwise. It should be noticed that the positions of the maxima and minima shift toward lower wavelengths as the films dehydrate with time or are squeezed tighter. This could be interpreted as a change in some periodic parameter in the super organization introduced by the twisting. It should be also noted in Fig. 2 that rotating the whole assembly by 90°, a test for linear dichroism artifacts (Eq. 7) that changes the signal shape little and only affects the values by lower magnitude effects, are probably caused by nonuniform thickness of the film.

Fig. 3 shows the CD of a film sandwiched between the two cuvettes as shown in Fig. 1c. The direct CD measured showed values of $\Delta \varepsilon = -260$ at 287 nm, and $-280$ at 232 nm. When the back scattering cone was measured by the use of the FDCD technique by placing the $\alpha$-napthylamine fluorescer in the back cuvette we obtained a remarkable back scattering CD signal with $\Delta \varepsilon = -120$. The front scattering measured by FDCD still gave a large value ($-160$). For the peak at around 230
the value either changed sign or was considerably reduced in magnitude. Unfortunately in this region the \( \alpha \)-napthylamine used in the FDCD measurement absorbs strongly so the signal to noise ratio is poor and consequently the measurement is not trustworthy. When a similar measurement of the back scattering cone is made for a different DNA film with counter-clockwise twist ("positive CD") by the mirror technique, we again obtained similar behavior. This film had values of \( \Delta \varepsilon = +540 \) at 284 nm and \(+321\) at 227 nm measured with regular CD (Fig. 4).

The back reflection mirror measurement shows a value of \( \Delta \varepsilon = +742 \) at 297 nm and \(+414\) in the 220-230 nm region. One must remember that these magnitudes are only for true scattering components and can be exaggerated by the denominator in Equation 6. It is obvious, however, that in these cases, at least for the CD signals in the 270-290 nm region, the back scattering components have the same polarization and approximately the same order of magnitude as the signals measured in the forward direction.

The behavior of the scattering components for the DNA film as a function of scattering angle are shown in Fig. 5. The CD shows a complicated behavior as the angle increases but in the region of the large minimum the sign remains negative. Thus in that region of the spectrum the scattered light shows essentially the same polarization as is measured in the "ordinary" CD measurement. Of interest is the CD scattering in the nonabsorbing regions, \( \lambda > 300 \) nm. These CD "tails" into the visible have been interpreted as loss of light of a particular polarization by deviation away from the detector (Dorman and Maestre, 1973). For scattering angles \( \theta = 0^\circ \) to \( \theta = 10^\circ \) we see
that there is a change in sign and this indicates that scattered light of the opposite polarization is being measured. Thus in regions of the spectrum away from the absorbance bands, the CD scattering can be a complicated function of the scattering angle. Similar behavior will be seen later in the CD scattering of DNA-polylysine aggregates.

Fig. 6 is a study of the variation of "normal" CD measurement as the angle of incidence of the light beam impinging upon the films is altered. These measurements were done to see if the behavior of the twisted DNA films in any way mimicked that of twisted nematics or cholesteric liquid crystal structure. For this particular film we found that a minimum in the magnitudes of the CD peaks occurred at about a ±20° tilting angle. This implies that there are certain directions in which "nulls" occur (i.e., the interaction with circularly polarized light is a minimum or is equal for both polarizations).

The above results have shown that a) the CD of twisted DNA films has very large magnitudes (i.e. large interactions), b) is dependent on the sense of the twist of the film, c) can be reversed by reversing the sense of the twist, and d) the CD signal and CD scattering signal are very sensitive to the orientation of the film vs the light beam and/or scattering angle of the light after interaction. In analogy to the optical properties of liquid crystals (cholesteric or twisted nematics) we propose the interpretation that the CD signals are a result of the long range order and organization of DNA closely packed in films or aggregates.
DNA-POLYLYSINE AGGREGATES EXPERIMENTS

Fig. 7 to Fig. 10 shows the CD scattering behavior of T7 phage DNA-polylsine and E. coli DNA-polylsine complexes at different stages of aggregation. Fig. 7 shows attempts at correction of the CD spectrum by FDCD methods. Presumably FDCD techniques as shown by Reich et al. (1979) will correct for all the scattering contribution that involve only deviation of the light away from the detector acceptance cone. It is shown that away from the negative CD band at 245 to 250 nm the FDCD correction does a fair job of eliminating the CD tails in the region between 270 nm to 290 nm. However, at the same time it increases the magnitude of the minimum from $\Delta \varepsilon(249 \text{ nm}) = -3.6$ to $\Delta \varepsilon = -5.0$. This is an analogous enhancement of the signal similar to the values reported in the EtOH-DNA particles study preceding this report. Fig. 8 shows two different preparations of T7 phage-DNA polylsine particles with the corrected FDCD spectra. The magnitude of the CD minima on those particles is about four times in value of that reported in Fig. 7. However, the CD tails are again corrected by the FDCD technique. Another type of particle, E. coli DNA-polylsine particle (Fig. 9), shows that its CD is essentially unchanged by the FDCD correction. It is evident from those particle studies that only a portion of the CD scattering pattern is "correctable" by large acceptance angle methods and that for those particles as well as the EtOH-DNA particles (Reich et al. 1979) a certain type of signal is not correctable and is even enhanced by increasing the collection efficiency of the detection system.

The CD scattering as a function of scattering angle $\theta$ (Fig. 10)
shows remarkably similar behavior to that of the DNA films (Fig. 5). The back scattering as measured by the mirror collection device (Fig. 1d) again shows the qualitative characteristics of the forward CD scattering or "normal" CD measurement.

Fig. 11 shows the CD scattering behavior of E-coli DNA-EtOH particles having extremely large CD values; i.e., ∆ε (normal, 275 nm) = +80. For such a large "resonance" CD the FDCD methods enhanced the CD at the maximum up to a value of ∆ε = +120.

One of the characteristics of the FDCD method is that the efficiency of correction increases as the total optical density of the solution reduced. In Fig. 11 a very low concentration of the DNA-EtOH particle solution is measured with a concomitant increase in noise in the signal. It can be seen that the magnitude of the signal does not change radically so that the CD spectrum at the resonance band is not a simple differential scattering signal but probably a manifestation of a liquid crystal (cholesteric or nematic) resonance reflection band having the associated anomalous transmission phenomenon.

In Fig. 11 it is also shown that the CD scattering at θ = 13° at the resonance band wavelength gives a scattering component having the same polarization as the forward scattering.
DISCUSSION AND CONCLUSIONS

We propose the thesis that the optical activity of the films, the DNA particles and the polylysine:DNA aggregates is a manifestation of a type of long range chiral asymmetry similar in nature to that found in cholesteric liquid crystals and twisted nematic liquid crystals.

The evidence for the above assertion is as follows:

a) Very large circular dichroism and ellipticities. In the films the circular dichroism ranges from $\varepsilon_r - \varepsilon_l = +800$ to $-220$, two orders of magnitude larger than the intrinsic magnitude of DNA solution. For DNA ethanolic condensates, we have shown values as large as $+150$. For DNA histone complexes values of the order of $\Delta\varepsilon = -80$ have been measured (Dorman; Maestre and Fasman, in preparation).

b) The sense of the measured optical activity can be directly correlated with the sense of the twist of the film. Moreover, for any given film this is a reversible phenomena; i.e., if the sense of the twist is reversed, so is the sign of the measured CD bands. Thus in Fig. 1 we show that a film with a counterclockwise twist of the top quartz plate, which produces a right handed helix, will produce large positive ellipticities at wavelengths, $\lambda_1 = 298$ nm and $\lambda_2 = 233$ nm. When this film is twisted in a reversed (clockwise) motion inducing a left handed helical twist or distortion we observe large negative values with minima at $\lambda_3 = 280$ nm and $\lambda_4 = 210$ nm.

This reversal of ellipticity can be produced several times until the orientation of the film is destroyed by the continual manipulation of the quartz plate.

c) The position of the CD maxima and minima of the films
seems to be related to their degree of hydration or thickness. As the films dry out or are squeezed mechanically so that they become thinner, the positions of the bands shift towards the blue; i.e., smaller wave length. Conversely, when the films are hydrated again the wavelength of the bands increase in value (red shift). This particular property mimics that found in cholesteric crystals in which a Bragg law behavior is seen at those wavelengths of circularly polarized light which match the periodicity and sense of helicity of the liquid crystal. For cholesterics or twisted nematics (Maugin limit) we have

\[ n\lambda = 2L \cos r' \]  

Eq. 8

where \( n \) = order, \( \lambda \) = wavelength at which the anomalous reflection or transmission occurs, \( L \) = periodicity of helix, and \( r' \) = the internal refractive angle (obtained from Snell's law). Thus for a beam at perpendicular incidence and \( n=1 \) (first order reflection) we conclude that at wavelength \( \lambda = 2L \) we should measure a very strong circular dichroism. However, in most of the CD spectra reported here we measure two bands. Even in the case where there is only a positive band, as in the DNA ethanolic particles, we see evidence of another band in the shorter region of the spectrum. It is probable that these two bands are a manifestation of two different modes of interaction of a large order helix with light. Furthermore, these bands show certain spatial distributions which also imply this interpretation, as we shall discuss below.

d) The DNA films also show a remarkable behavior as a function of angle of incidence of the light beam. As shown in Fig. 6, as the film is tilted with respect to the light beam incidence we go through a minimum angle at \( \pm 20^\circ \) from the vertical in which the first negative
peak at $\lambda = 270$ nm disappears. As the angle is increased we get a recovery of the CD peak. This is the kind of optical behavior that is seen in cholesteric liquid crystals with a well defined structure and is interpreted as a shift in critical wave length outside of the absorbance region produced by the Bragg law behavior.

The scattering behavior of the films, the DNA-EtOH particles and the nucleohistone-DNA and polylysine-DNA particles show that the measured CD is mainly a scattering resonance phenomenon that occurs at certain critical wave lengths. In particular the measurement of the back scattering regions of the film show magnitudes of the same order as those measured in the forward direction. At the critical wavelengths the circular polarization of the scattering seems to remain constant as a function of scattering angle. However, in the region of the spectrum away from the critical wavelength we observe a reversal of the sense of circular polarization. Thus, different regions of the CD spectrum of these structures behave with a different spatial distribution which indicates that they are produced by different scattering mechanisms.

e) What is most remarkable is the large increase in the magnitude of some of the CD curves at the critical wavelength when attempts are made to recover all of the scattered light by large angle integrating methods such as FDCD or fluorescent cells. It is as if more light is coming out of the system at a given critical wavelength than is being put in. Thus, when the scattered light is measured over a sphere of $4\pi$ steradians there is a sudden increase in the measured radiation at the critical wavelength. A similar mechanism exists in cholesteric liquid crystals analogous to the Bormann effect (1941) for x-rays,
and investigated by Nitayananda (1973) and Luresh (1976). This can be viewed as an anomalous transmission phenomenon.

In our particular case we propose the following mechanism for the critical wavelengths: The light is absorbed by the normal electronic transition in the nucleic acid bases of the DNA. However, this energy which is usually lost by radiationless transition processes in the normal DNA structure is now coupled to the helical super organization matching the polarization and repeat periodicity of the incoming light beam. In analogy to a cholesteric liquid crystal this will cause a resonance phenomenon such that the absorbed energy is re-radiated in particular directions with the same polarization and wavelength as the incoming light beam. This mechanism is well known in the interaction of helical antennae with circularly polarized radiowaves. A model system of optical activity was studied by Freeman and Tinoco (1957) using helical coils of copper wire and a microwave generator. These workers found that the coils showed strong cotton effects at wavelengths determined by the periodicity of the wire helix and given by the equation \( n\lambda \approx 2L \) (where \( L \) is a linear function of the repeat period of the wire coil). There were two circular dichroic bands, one at the axial mode of interaction of the coil ("end fire" mode in antenna theory, see Krauss, 1950) and the other at shorter wavelength at the normal mode of oscillation of the coil.

Fig. 3 shows the back scattering cone as measured by FDCD and Fig. 4 shows it as measured by the mirror back collection device. In both cases the higher wavelength CD peaks have the same polarization as the CD peak measured in the forward direction. The short wavelength
peak is either absent or suppressed in magnitude. We are led to the assumption that this mode is probably oscillating in a perpendicular direction to the light beam and thus is weakly detected by back scattering detection devices.

Therefore, because of reasons enumerated above we have concluded that the extremely large CD values reported for films, nucleohistones and certain types of condensed DNA at specific wavelengths reflect a certain long range order of a helical nature. The sense of the helicity is reflected in the sign of the so-called psi spectra (i.e., a positive CD reflects a right handed helical arrangement and a negative CD reflects a left handed coil). Furthermore, the positions of these large peaks reflect the repeat periodicity and are probably given by a Bragg reflection law analogous to the law for cholesteric liquid crystals, Eq. 8, or to that obtained for helical antenna arrays (Krauss, 1950).

\[ n \lambda = 2L \quad \text{axial mode} \quad \text{Eq. (9)} \]

or \[ n \lambda = 2d \quad \text{normal mode} \]

\( L = \) helical periodicity, \( d = \) diameter of helix.

It is important to realize that it is the helical ordering that provides the mechanism for the large CD values. Any random packing of nucleic acids will not produce any change in CD except that produced by changes in secondary structure. Gosule and Schellman (1976) have reported DNA-putrescine and DNA-spermine aggregates of very defined morphological sizes in which no changes in CD were detected. Similarly Tunis-Schneider and Maestre (1970) have shown that films prepared in the proper way show little difference from the CD of DNA and RNA. We
have found that it is easy to change one of the psi type films to obtain the regular conservative CD spectra of DNA by the proper manipulation of the quartz plates.

It is also important to note that the secondary structure of the DNA has little or nothing to do with the psi type spectra. It may be possible to correct for the psi spectra by tilting the films until a null angle is obtained and then measure the remaining spectra which are presumably caused by the secondary structure, but this is a difficult experimental problem. In particles in solution this will be possible only if the psi type bands are fortuitously far away from some of the significant CD spectrum regions of the DNA which reflect secondary structures (for example, the 270 - 290 nm region).

The last remaining topic of discussion concerns those CD scattering perturbations that can be corrected by integrating methods; e.g., the long tails outside of the absorbance regions of the spectrum. These tails are also measured in the CD spectrum of cholesteric crystals (Holzwarth and Holzwarth, 1973). Invariably the tails can be corrected by FDCD or Fluorscat cell methods indicating that the light is lost by differential scattering (i.e., one polarization is deviated more from the optical axis than the other). The explanation for this behavior is reasonably straightforward. It is a manifestation of the entire size and shape of the particle (i.e., Mie or "form" type of scattering) but in this case differential scattering occurs for different polarizations. This is also true for bacteriophage T2 and T4 (Dorman and Maestre 1975, Holzwarth et al. 1976). These tails were shown to be proportional to the Kronig-Kramers transforms of the Fluorscat corrected signal.
of the psi type spectrum. Therefore, in this sense it is a true Mie type optically active or "form" dichroic scattering phenomenon. The DNA-EtOH particles show a more complicated behavior since for this type of particle the corrected scattering is at right angles to the beam. This is probably a reflection of the smaller size of the particle obtained for this particular set of measurements. We did not measure a large back scattering reflection with FDCD and the mirror device for these particles; thus most of the scattering components are probably perpendicular to the light beam. Therefore this type of "correctable" CD scattering is a reflection of index of refraction dispersion and the bulk size, shape and sense of helicity of the particle.

The nature of this type of scattering is analogous to that of form birefringence manifested by arrays of rods or plates when studied with linearly polarized light (Wiener, 1926). In this case the form effect is a circular birefringence form effect which differentiates between right handed and left handed helical structure.

It should be noted that in the case of the polylysine T-7 DNA condensates (Fig. 10) there is a strong back scattering component; therefore the resonance mode of liquid crystal type scattering points to a different organization than that of DNA-EtOH condensates.
CONCLUSIONS

From the studies of the CD and the scattering differential CD of films of DNA with twisted helical structure, polylysine-DNA psi type particles (negative) and DNA-EtOH salt aggregates (positive \( \psi \) spectra) we have arrived at the following conclusions:

a) Psi type CD spectra are mainly a manifestation of resonance scattering phenomena with an associated anomalous transmission in the absorbing bands.

b) The CD bands outside of the absorbing region can be corrected by the use of devices that increase the collection geometry such as Fluorscat cells and FDCD methods. This is the birefringent dispersion component and is not only a function of the bulk index of refraction but reflects the size and shape of the particle as pointed out by Holzwarth and Holzwarth (1973), and Holzwarth et al. (1974).

c) We have shown that the CD scattering of these particles can be divided into two types which reflect the different properties of the scattering structure. Information can be obtained on the long range organization by demonstrating that some of the scattering exhibits optically active behavior such as is found in cholesteric crystals or twisted nematic crystals. In such cases the sense of twist or super helix can be determined from the sign of the CD bands. A right handed helix gives positive CD signals and a left handed helix gives negative CD signals. The periodicity is given by the Bragg law: \( n\lambda = 2L \cos r \). Thus for a typical psi (-) nucleohistone aggregate which exhibits a critical \( \lambda = 270-280 \) nm, the helical periodicities would be on the order of 1350 to 1400 \( \text{Å} \). As indicated in the experimental results
it is probable that DNA molecules stacked side by side and dehydrated will twist from a parallel arrangement to a left twisted arrangement as they dehydrate and come closer together.

e) In the few cases in which correction can be obtained at low salt concentration for DNA-polylysine complexes we find that the corrected CD spectrum has a depressed band in the 270-290 nm region indicating that this salt concentration the secondary structure of the DNA is changing towards the C form geometry. However, since the resonance bands increase in magnitude it is impossible to obtain any information concerning the secondary structure of these molecules. It would be possible to measure such information if some type of quasi-liquid crystal bulk organization (such as a film) could be obtained and then measurement could be performed at certain null angles given by the Bragg reflection law for liquid crystals.

f) A model of the geometry of DNA in these aggregates is shown in Fig. 12. By a helical array we mean a parallel organization of DNA molecules which are then twisted slightly so that each layer of DNA molecules is at a slight angle of twist with respect to the two neighboring ones. Each layer of DNA would be then twisted with respect to the two neighboring layers in a manner similar to that found in cholesteric liquid crystals or twisted nematic liquid crystals. The long range order of these layers would then provide the necessary helical periodicity to produce the strong resonance bands measured by circular dichroism. The planes in the figure are placed far apart for clarity but in the DNA film we assume that these planes are in proximal contact. Indeed from our experiment this contact seems to be so close as to bias the
twist in only one direction since DNA films tend to give negative psi type spectra if not forced into a given twist mechanically or forcibly disoriented. Therefore it is probable that some type of minimum energy configuration exists between quasi-parallel arrangements of DNA molecules that imposes a long range left-handed organization as in Figure 12(b).

g) The above would explain the behavior of condensed DNA in bacteriophage T2, T4, T6 in which an apparent liquid crystalline component negative in value and centered at 260 nm is obtained (Holzwarth et al. 1974). The periodicity for the condensed T2 DNA package then would be of the order of 1300 Å if we assume that it obeys the Bragg scattering law, Eq. (8), for a first order reflection. The CD tails would also be a property of the size of the particle since they are caused by birefringence dispersion. Smaller bacteriophage such as T5 and T7 (Maestre et al. 1971) show a much smaller contribution to the CD tails.
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FIGURE LEGEND

Fig. 1. Schematics of the differing techniques and devices used in the measurement of the CD scattering components of films, DNA-polylysine particles and DNA-ETOH condensates. All these devices were used in a modified Cary 60 dichrograph.

Fig. 2. CD of DNA films measured in a vertical CD assembly as a function of twist of film and orientation of film about the optical axis of the light beam. Twisting occurred in the sequential order described in curve labels.

Fig. 3. DNA film with clockwise twist producing a negative \( \psi (-) \) CD spectrum. This film was produced between two cuvettes as described in Figure 1c to enable measurement of "normal" CD. Forward cone (forward cell full of fluorescer in FDCD mode) and back cone (back cell full of fluorescer in FDCD mode). Note that scattered light is equivalent to transmitted light, and since the CD instrument measures \( (T_r - T_l) \), the curve labeled FDCD back scatter is \( (A_{sr} - A_{sl}) = -A_s \) as determined by the equations in the appendix.

Fig. 4. DNA film with counterclockwise twist measured by normal CD and the back mirror device as in Fig. 1d. Notice the polarization remains essentially the same throughout the spectrum.

Fig. 5. DNA film with counterclockwise twist as a function of scattering angle. The goniometer device is sketched in Fig. 1a.

Fig. 6. DNA film normal CD as a function of changing incidence angle of light beam. This is measured in the vertical mode device.

Fig. 7. T7 phage DNA-polylysine complexes produced in different
salt concentrations. The FDCD correction is achieved by the device shown in Fig. 1b.

Fig. 8. T7 DNA polylysine particles with large CD negative bands. Note that the two different preparations have different minima in the uncorrected CD spectra. Upon correction the CD is very similar.

Fig. 9. E. coli DNA-polylysine complex particles. For these particles the correctable scattering contribution using the FDCD method is minimal.

Fig. 10. The E. coli-DNA polylysine particles of Fig. 9 were measured by the goniometer device (Fig. 1a) to determine the characteristics of the CD spectrum scattering. A comparison with film scattering (Fig. 5) shows very similar behavior qualitatively although the magnitudes are smaller in the particle case. The curve with crosses (xxxx) shows the back scattering obtained with the mirror device shown in Fig. 1(d).

Fig. 11. FDCD corrections of DNA-EtOH particles as a function of optical density of the sample. Also shown is normal CD and CD scattering at $\theta = 13^\circ$ scattering angle.

Fig. 12. Proposed model of DNA packing in films and aggregates. Figs. 12(a) and 12(b) show the sense imposed upon the film by the mechanical twisting. Fig. 12(c) represents the actual interpretation given to twisted nematic and cholesteric liquid crystals applied to the DNA fibers in a twisted DNA film.
APPENDIX

DNA FILM SCATTERING

The assumption is made here that the film is thin compared to the wavelength of light so that it can be considered to be two-dimensional with no thickness. The experimental set-up is as in Fig. 1(d). The front cuvette is on the left before the DNA film. The back cuvette is on the right after the DNA film. The light beam travels from left to right in Fig. 1(d).

\[ A_F = \text{absorption of the fluorescent solution in one of the cuvettes; i.e., } 0.2 \varepsilon_F C_F. \]

\[ A_{DL,R} = \text{absorption of the DNA film for left (L) and right (R) circularly polarized light.} \]

\[ A = A_F + A_D; A_L = A_F + A_{DL}; A_R = A_F + A_{DR}; \]

\[ \theta_F = \text{FDGD ellipticity} \]

I. Suppose film does not scatter and both cuvettes are filled:

Intensity of light absorbed in first cuvette by fluorescer

\[ (1) \quad I_1 = I_0(1 - e^{-2.303A_F}) \]

Intensity of light reaching DNA film is:

\[ (2) \quad I' = I_0 e^{-2.303A_F} \]

Intensity of light after going through film is:

\[ (3) \quad I'_0 = I'e^{-2.303A_D} = I_0 e^{-2.303A} \]

Intensity of light absorbed in second cuvette is:

\[ (4) \quad I_2 = I_0(1 - e^{-2.303A_F}) = I_0 e^{-2.303A(1 - e^{-2.303A_F})} \]
Total intensity of light absorbed by the fluorescer solution for left circularly polarized light is:

\[
(5) \quad I_L = I_{IL} + I_{2L} = [I_{OL} + I_{0L} e^{-2.303A_L}] [1 - e^{2.303A_F}]
\]

\[
(6) \quad I_R = I_{1R} + I_{2R} = [I_{OR} + I_{0R} e^{-2.303A_R}] [1 - e^{-2.303A_F}]
\]

\[
(7) \quad \theta_F = k \left( e^{-2.303A_L} - e^{-2.303A_R} \right) \frac{[1 - e^{-2.303A_F}]}{(2 + e^{-2.303A_L} + e^{-2.303A_R}) [1 - e^{-2.303A_F}]}
\]

where \( A = (A_L + A_R)/2 \)

\[
(8) \quad \theta_F = k \frac{10^{-A_L} - 10^{-A_R}}{2 + 10^{-A_L} + 10^{-A_R}} \quad \text{(multiply num. and denom. by } 10^A) \]

where \( \Delta A = A_L - A_R \)

\[
(9) \quad \theta_F = k \frac{10^{-\Delta A/2} - 10^{+\Delta A/2}}{2 \cdot 10^A + 10^{-\Delta A/2} + 10^{+\Delta A/2}} \quad \text{(expand around } \Delta A) \]

\[
(10) \quad \theta_F = k(-2.303\Delta A) \quad \text{where } k = -28.65
\]

\[
(11) \quad \theta_F = \frac{(14.32)(2.303)\Delta A}{10^A + 1}
\]

\[
(12) \quad \Delta A = \frac{\theta_F(10^A+1)}{(32.98)}
\]

II. Suppose film back scatters 180° totally (i.e., beam reverses upon hitting DNA film). It can easily be seen that since the film is
between two cuvettes of equal dimensions, the absorption of the back scattered light is exactly the same as the forward unscattered light and, thus, Eq. (12) holds for this case also.

III. Suppose only the first or front cuvette is filled and the DNA film has a 180° component of back scattering. The second cuvette or back cuvette is empty. \( A_s \) = apparent absorption due to back scattering.

Intensity of light scattered by DNA film is:

\[
I_{os} = \frac{A_s}{A_D} I'(1 - e^{-2.303A_D})
\]

\[
= \frac{A_s}{A_D} I_o (1 - e^{-2.303A_D})e^{-2.303A_F}
\]

Intensity of scattered light reaching L is:

\[
I_s = I_{os} e^{-2.303\varepsilon_F F(d-l)}
\]

Intensity of scattered light absorbed by L is:

\[
dI_s = 2.303\varepsilon_F F d I_s d\ell
\]

\[
= e^{-2.303A_F} I_o \frac{2.303\varepsilon_F F A_s}{A_D} (1 - e^{-2.303A_D})
\]

\[
x[e^{-2.303\varepsilon_F F (d-l)}] d\ell
\]

Total intensity of scattered light absorbed is:

\[
I_s' = \left[ \frac{2.303\varepsilon_F F A_s}{A_D} I_o e^{-2.303A_F} (1 - e^{-2.303A_D})e^{-2.303\varepsilon_F F d} \right]
\]
\[
x \int_0^d e^{2.303 \epsilon_F r \delta d \ell}
\]

\[= I_{os} e^{-2.303AF}(e^{2.303AF} - 1)\]

(16) \[I_s' = I_{os}(1 - e^{-2.303AF}) \text{ which is expected.}\]

(17) \[I_s' = \frac{A_s}{A_D} (1 - e^{-2.303AD})(1 - e^{-2.303AF})e^{-2.303AF}\]

\[I_L = \text{total intensity of left cpl absorbed by fluorescer}\]

\[I_R = \text{total intensity of right cpl absorbed by fluorescer}\]

(18) \[I_L = (I_o + I_{osL})(1 - e^{-2.303AF})\]

\[= [I_o + I_o \frac{A_{SL}}{A_{DL}} (1 - e^{2.303A_{DL}L})e^{-2.303AF}](1 - e^{-2.303AF})\]

\[\therefore \]

(20)

\[
\theta_F = k \cdot \frac{\frac{A_{SL}}{A_{DL}} (1 - e^{-2.303A_{DL}L})e^{-2.303AF} - \frac{A_{SR}}{A_{DR}} e^{-2.303AF}(1 - e^{-2.303A_{DR}R})}{2 + \frac{A_{SL}}{A_{DL}} e^{-2.303AF}(1 - e^{-2.303A_{DL}L}) + \frac{A_{SR}}{A_{DR}} (1 - e^{-2.303A_{DR}R})e^{-2.303AF}}
\]

for: \[A_D = (A_{DL} + A_{DR})/2 = \text{DNA absorption}\]

\[\Delta A_S \equiv A_{SL} - A_{SR} = \text{differential back scattering}\]

\[\Delta A \equiv A_{DL} - A_{DR} = \text{differential DNA absorption}\]

\[A_S \equiv (A_{SL} + A_{SR})/2 \quad \text{total scattering in back direction.}\]
then
\[ \theta_F = k \cdot \frac{A_{SL} A_{DR} - A_{SR} A_{DL}}{A_{DL} A_{DR}} (10^{A_D} - 1) + \frac{2 \cdot 3.303 \Delta A}{2} \frac{A_{SL} A_{DR} + A_{SR} A_{DL}}{A_{DL} A_{DR}} \]

\[ 2 \cdot 10^A + \frac{A_{SL} A_{DR} + A_{SR} A_{DL}}{A_{DL} A_{DR}} (10^{A_D} - 1) + \frac{2 \cdot 3.303 \Delta A}{2} \frac{A_{SL} A_{DR} - A_{SR} A_{DL}}{A_{DL} A_{DR}} \]

Note: \( A_{DL} \approx A_{DR} \approx A_D \). Then we get approximation

\[ \theta_F = k \cdot \frac{\Delta A_S - A_{SR} \Delta A}{A_{DL} A_{DR}} (10^{A_D} - 1) + \frac{2 \cdot 3.303 \Delta A}{2} \frac{A_{SR} \Delta A}{A_{DL} A_{DR}} \]

Assume \( A_S \) is small compared to total absorption \( A_D \)
(Note \( A_S \) is not total scattering but back scattering, i.e. measured by the front cuvette).

(23) \[ \theta_F = k \cdot \frac{\Delta A_S}{A_{DL}} (10^{A_D} - 1)/2 \cdot 10^A \]

\[ = k \frac{\Delta A_S}{A_{DL}} (10^{-A_F} - 10^{-A}) \]

\[ \approx -14.32 \frac{\Delta A_S}{A_D} (10^{-A_F} - 10^{-A}) \]

(24) \[ \Delta A_S = -\frac{\theta_F A_D}{14.32 (10^{-A_F} - 10^{-A})} \]
0

C'ANGLE OF TILT OR
INCIDENCE ANGLE

8' SCATTERING
ANGLE

8' ORIENTATION ANGLE ABOUT
OPTICAL AXIS OF LIGHT BEAM

SLITS
PHOTOMULTIPLIER
(1/8" DIAMETER
HAMAMATSU #376)

BEAM GONIOMETER MOUNT

FIG. 1a

FIG. 1b

FDGD DEVICE

BACK CUVETTE
LIGHT BEAM
DNA FILM
UV FILTER
#375 PM TUBE

FDGD BACK REFLECTION DEVICE

FIG. 1c

BACK REFLECTION MIRROR DEVICE

FIG. 1d

Cuvette
LIGHT BEAM
RAYLEIGH HORN
UV FILTER
2" HAMAMATSU #375 PM TUBE

DNA FILM OR CUVETTES

FRONT SURFACE MIRROR

SLIT

1/8" HAMAMATSU #376 PM TUBE
The graph depicts the difference in molar absorptivities (ε_L - ε_R) against wavelength (nanometers) for various treatments:

- **NORMAL CD**
- **FOCD CORR.**
- **FOCD BACK SCATT.**

The wavelength range is from 200 to 340 nanometers, with specific peaks and troughs indicative of the absorption properties at different wavelengths.
\[ \epsilon_L - \epsilon_R \]

Wavelength (nanometers)

- NORMAL CD
- MIRROR BACK SCATT.

XBL796-3551
17 DNA-POLYLYSINE COMPLEXES

![Graph showing UV spectra of DNA-polylysine complexes. The x-axis represents wavelength (nanometers) from 210 to 350, and the y-axis represents epsilon left - epsilon right from -4.0 to 1.0. The graph includes lines for CD 0.85M salt, FOCO CORR. 0.85M salt, CD 1M salt, and FOCO CORR. 1M salt.](image-url)
T7 DNA-POLYLYSINE COMPLEXES, .15M SALT

CONVENTIONAL CD
FOCD CORR.
CONVENTIONAL CD
FOCD CORR.

WAVELENGTH (NANOMETERS)
E. COLI DNA-POLYLYSINE GONIOMETER CD

0 DEGREES
13 DEGREES
23 DEGREES
33 DEGREES
BACK SCATT. MIRR or X .5

WAVELENGTH (NANOMETERS)

0
5.0
10.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
Counterclockwise twist in films

DNA molecules

Repeat periodicity

Axis of light propagation

Clockwise twist

DNA axis

Left Handed Array Organization (b)

Right Handed Array Organization (a)

2nd Layer + twist

1st Layer of DNA molecules

DNA molecule

Optical axis

Image XBL7811-3693
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